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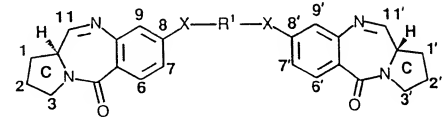

(I)

(57) Abstract

There are disclosed, as novel compounds, pyrrolo [2,1-c][1,4] benzodiazepine (PBD) dimers of formula (I) where the PBD tricyclic structures may contain additional substituents in one or more of the 1, 2, 3, 6, 7, 9 and 11-positions and the C rings may optionally contain at least one further hetero ring atom, X is -O-, -S- or -NH-, and R^1 is an alkylene chain containing from 3 to 12 carbon atoms, which chain may, if desired, be interrupted by one or more hetero-atoms and which chain may, if desired, contain one or more carbon-carbon double or triple bonds. These compounds are cytotoxic and have shown *in vitro* activity against a range of carcinoma cell lines.

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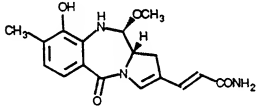
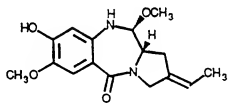
Anti-cancer pyrrolobenzodiazepine derivatives

This invention is concerned with certain novel pyrrolobenzodiazepine derivatives, with a method for their preparation and with pharmaceutical compositions containing them.

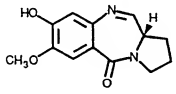
A group of pyrrolo[2,1-c][1,4]benzodiazepines (PBDs) are known antitumour antibiotics. These compounds are produced by various Streptomyces species and well-known members of this group include anthramycin, tomaymycin, DC-81, neothramycin and sibiromycin:



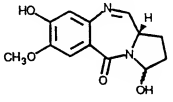
ANTHRAMYCIN



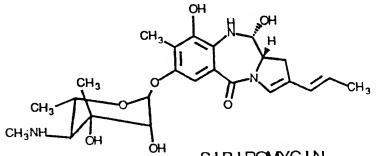
TOMAYMYCIN



DC-81



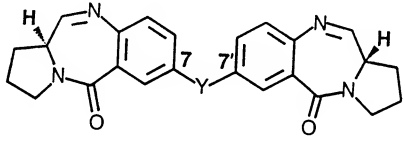
NEOTHRAMYCIN



SIBIROMYCIN

see Remers, W.A.: Pyrrolo[1,4]benzodiazepines, The Chemistry of Antitumour Antibiotics, Vol.2, pp.28-92, Wiley-Interscience (1988). Their antitumour and antibacterial activity are thought to be a direct consequence of DNA-binding which may interfere with processes such as DNA replication or translation.

J.D. Farmer, Jr. et al, Tetrahedron Letters, Vol 29, No. 40, pp 5105-5108, 1988 have described the synthesis of a dimeric anthramycin analogue and its use to effect DNA-crosslinking. The Farmer et al paper discloses three dimers, that is compounds of the formula:



in which Y is, respectively, $-S(CH_2)_6S-$, $-O(CH_2)_2-N(CH_3)(CH_2)_2O-$ and $-HN(CH_2)_3N(CH_3)(CH_2)_3NH-$; it appears that the third of these compounds was not actually obtained by the synthesis described. The DNA cross-linking ability of the second compound, with the N-interrupted diether linkage, was examined. In order to obtain cross-linking, the dimer was heated with DNA at 65°C for 1 hour. An alkaline agarose gel assay (under conditions which protected the DNA) of the dimer-cross-linked DNA against uncross-linked DNA and psoralen-cross-linked DNA, showed that the DNA had been cross-linked by the dimer.

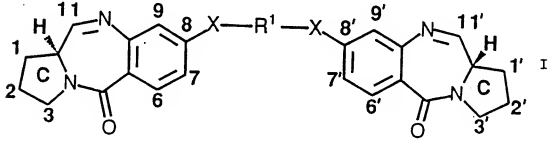
This cross-linking could be reversed, that is the dimer could be released from the DNA, by incubating the cross-linked DNA at a pH of 10 for 12 hours at 25°C.

It will be noted that the Farmer et al dimers

are linked through the 7-positions of the anthramycin tricyclic structures.

We have now found that PBD dimers having a linking group attached to the 8-positions of the PBD structures are capable of cross-linking double-stranded DNA significantly more readily than the dimers described in the Farmer et al paper. Our novel dimers have isohelicity with DNA and are capable of fitting snugly into the minor groove of the latter.

According to one aspect of the present invention, there are provided, as novel compounds, pyrrolo[2,1-c][1,4]benzodiazepine (PBD) dimers of the formula;



I

where the PBD tricyclic structures may contain additional substituents in one or more of the 1, 2, 3, 6, 7, 9, and 11-positions and the C rings may optionally contain at least one further hetero ring atom, X is -O-, -S- or -NH-, and R^1 is an alkylene (i.e. polymethylene) chain containing from 3 to 12 carbon atoms, which chain may, if desired, be interrupted by one or more hetero-atoms, and which chain may, if desired, contain one or more carbon-carbon double or triple bonds.

Suitable substituents in the tricyclic rings include, for example, hydroxyl, alkoxy, such as methoxy, ethylidene and 2-acrylamidyl groups. Another preferred

group of substituents have the formula:

$$-O(CH_2)_n-NH_3^+Cl^-$$

where n is an integer from 1 to 6, and are substituted in the 2- and 2'- positions; it is generally required that the compounds according to the invention should be water soluble and such substituents, and also hydroxyl substituents in the 2- and 2'- positions, have a substantial solubilising effect.

Preferred compounds according to the invention are those in which the PBD structures have: methoxy substituents in the 7- and 7'- positions (derived from DC-81); methoxy substituents in the 7-, 7'-, 11- and 11'- positions and ethylidene substituents in the 2- and 2'- positions (derived from Tomaymycin); and hydroxyl substituents in the 9- and 9'- positions, methoxy substituents in the 11- and 11'- positions, and 2-acrylamidyl substituents in the 2- and 2'- positions (related to Anthramycin).

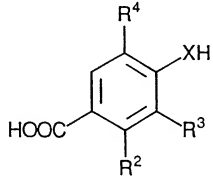
Preferred linking groups are those in which X is -O- and R^1 is an alkylene chain containing from 3 to 6 carbon atoms or a chain of the formula $-(CH_2)_x-O-(CH_2)_x-$, where x is an integer of from 2 to 6, or a chain of the formula $-(CH_2)_n-CH=CH-(CH_2)_n-$, where n is an integer from 1 to 5.

Whilst it has been demonstrated that a compound according to the invention in which R^1 is propylene, i.e. $-CH_2.CH_2.CH_2-$, effectively cross-links the two strands of double helical DNA, that is it effects interstrand cross-linking, it is believed that compounds according to the invention in which the R^1 chain is of suitable length and contains at least one rigid linkage, that is a double or triple bond, may be capable of cross-linking two molecules of double-stranded DNA (i.e. intermolecular cross-linking).

According to another aspect of the present

invention, there is provided a method of preparing a PBD dimer of formula I, which comprises:

(i) condensing a benzoic acid derivative of the formula:

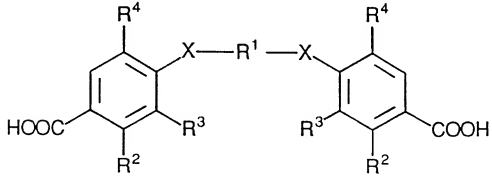


II

where X has the above-stated meaning (i.e. -O-, -S- or -NH-), R^2 is H or a desired substituent in the 6- or 6'-position of the PBD dimer, R^3 is H or a desired substituent in the 7- or 7'- position of the PBD dimer, and R^4 is H or a desired substituent in the 9- or 9'-position of the PBD dimer, with a dihaloalkane derivative of the formula:

$$X\text{-}R^1\text{-}X \qquad \text{III}$$

where R^1 has the above-stated meaning and X is a halogen atom (preferably iodine), by adding the dihaloalkane III slowly to a reaction mixture containing the benzoic acid derivative II to form a dimer acid compound of the formula:

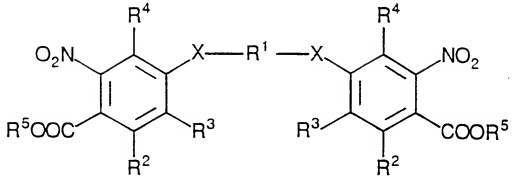


IV

(ii) esterifying the dimer acid of formula IV,

(iii) nitrating the diester from step (ii) to

obtain a dinitro-diester compound of the formula:

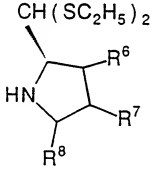


V

where R^5 is an alkyl group,

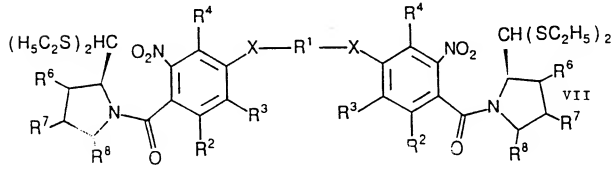
(iv) hydrolysing the dinitro-diester compound of formula V to remove the esterifying groups and obtain the corresponding dinitro-diacid compound,

(v) condensing the dinitro-diacid compound from step (iv) with a (2S)-pyrrolidine-2-carbaldehyde diethyl thioacetal of the formula:



VI

where R^6 is H or a desired substituent in the 1- or 1'-position of the PBD dimer, R^7 is H or a desired substituent in the 2- or 2'- position of the PBD dimer, and R^8 is H or a desired substituent in the 3- or 3'-position of the PBD dimer, to form a nitrothioacetal derivative of the formula:



VII

(vi) reducing the nitro substituents of the derivative of formula VII to amino groups, and

(vii) subjecting the diamino compound from step (vi) to ring closing conditions whereby a PBD dimer of formula I is obtained.

The steps of the process according to the invention and the preferred reactants and reaction conditions for use therein, will now be described in greater detail.

(i) The starting materials for this step, that is the benzoic acid derivatives II and the dihaloalkane derivatives III are commercially available or can be prepared by routine procedures from commercially available compounds. The dihaloalkane derivatives III include, of course, compounds in which the alkane chain is interrupted by one or more heteroatoms or by one or more double or triple bonds. Precursors of these compounds are commercially available, for example $HO-(CH_2)_x-O-(CH_2)_x-OH$ and $HO-(CH_2)_n-CH=CH-(CH_2)_n-OH$, and these can be readily converted to the corresponding dihalo compounds by standard procedures.

The condensation reaction of step (i) is preferably carried out in a two-phase reaction medium, that is a mixture of an organic solvent, such as tetrahydrofuran, in which both the reactants II and III are soluble, and an aqueous alkali, such as aqueous sodium hydroxide, a solution of the dihaloalkane III in the organic solvent being added slowly to the reaction medium which already contains the benzoic acid derivative II. We have found that by operating in this way the production of monoalkylation and elimination products (arising from the loss of HX) is minimised.

The reaction is preferably carried out at an elevated temperature, for example from 60°C to the reflux temperature of the reaction mixture.

(ii) The esterification may be carried out by any of the conventional procedures. A preferred method involves initial formation of the acid chloride using either oxalyl chloride or thionyl chloride in an inert solvent, such as tetrahydrofuran (THF) or benzene, followed by treatment with an excess of methanol to form the methyl ester. The use of other alcohols, such as ethyl or propyl alcohol, would form the corresponding ethyl or propyl esters.

Other methods for converting aromatic acids to the corresponding esters include the use of dimethyl sulphate/potassium carbonate/acetone which normally produces high yields of methyl esters in one step. A solution of diazomethane (CH_2N_2) gas in a solvent, such as ether, will also convert aromatic acids directly to the methyl ester. A conventional method of ester synthesis involves refluxing the aromatic acid with methanol or another appropriate alcohol in the presence of an acid catalyst, such as sulphuric acid. A number of more recent and more novel reagents for ester formation are described on pages 154-156 of the well-known book "Protective Groups in Organic Synthesis" by Theodora W. Greene (John Wiley & Sons, 1981).

(iii) Nitration of the product of step (ii) may be effected by any of the standard procedures. A preferred method comprises the use of stannic chloride ($SnCl_4$) and fuming nitric acid in methylene chloride at -20°C.

A simple nitrating mixture of H_2SO_4/HNO_3 or $NaNO_2/H_2SO_4$ can also be used. Other nitronium salts, such as $NO_2^+PF_6^-$, can also be used and these are described in a paper by Effenberger and Geke, Synthesis, 40 (1975).

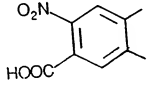
(iv) Hydrolysis of the product of step (iii) may be carried out by any of the standard procedures. A preferred method comprises aqueous sodium hydroxide in

THF at room temperature.

Lithium hydroxide in aqueous methanol can also be used. Other methods include the use of a nucleophile, such as lithium chloride, in an aprotic solvent (eg DMF). A mixture of $(CH_3)_3SiCl/NaI$ in CH_3CN can also effectively cleave esters. KO_2/18-crown-6 in benzene and DBN (1,5-diazabicyclo[4.3.0]non-5-ene)/xylene can also be used. These methods and others are described in detail on pages 156-159 of the book by Theodora W. Greene referred to above.

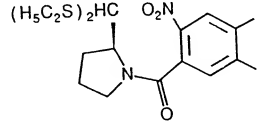
(v) The (2S)-pyrrolidine-2-carbaldehyde diethyl thioacetal VII required for this step may be prepared in five steps from L-proline or a suitably substituted proline by the method of Langley and Thurston, J. Org. Chem., 52, 91 (1987).

The condensation of the thioacetal VI with o-nitrobenzoic acid derivatives of the formula:



VIII

to form PBD precursors of the formula:



IX

has been described in said Langley and Thurston paper and the condensation of step (v) is preferably carried out under the same conditions, that is the addition of the acid chloride to a suspension of the diethyl-thioacetal and triethylamine in water. Another method involves the use of acetonitrile as solvent in which case the water can be omitted.

(vi) The reduction of the nitro groups of

the product of step (v) to amino groups may be carried out by any of the standard procedures. A preferred method uses stannous chloride dihydrate ($SnCl_2.2H_2O$) in methanol.

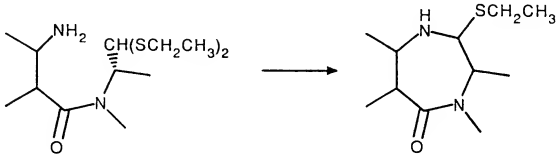
Other chemical reducing agents which can be used include, for example, iron/acetic acid/methanol, sodium dithionite ($Na_2S_2O_3$) in methanol, and iron carbonyl ($Fe_3(CO)_{12}$). Sodium borohydride combinations, such as $NaBH_4$/i-PrOH or $NaBH_4$/Pd-C, can also be used.

(vii) The final cyclization step is similar to the cyclization step for making PBDs described by Langley and Thurston, J. Org. Chem., 52, 91-97 (1987) and is preferably carried out under the same conditions, that is in the presence of mercuric chloride ($HgCl_2$) and calcium carbonate ($CaCO_3$) in a mixture of acetonitrile and water (preferably 4:1 by volume). We have found that the reaction is complete in about 2.5 hours, as opposed to the 24 hours reported in the paper just referred to.

We have also found that it is desirable to use a different working up procedure from that described in the Langley and Thurston paper. On conclusion of the reaction, the solvents are removed by evaporation and the residue is filtered and/or directly loaded on to a silica gel column which is first eluted with ethyl acetate to remove mercuric salts. Further gradient elution with 3%-5% methanol in chloroform (HPLC grade) allows the product to be eluted in very high yields (80% or more compared with 15% or less using the work-up procedure of the paper).

In a modification of the final cyclization step, the diaminothioacetal compound from step (vi) is reacted with sulphuryl chloride (SO_2Cl_2) in the presence of wet silica gel in a suitable organic

solvent, such as dichloromethane. This causes ring closure with the formation of an aminothioether structure in the 10,11- positions, that is



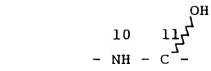
These 11- and 11'- thioether compounds are preferred compounds according to the invention and may be isolated and purified as such.

Alternatively they may be further treated to obtain the imine structure, -NH=CH-, in the 10, 11- positions by reaction with mercuric chloride or silver nitrate in a suitable solvent, for example aqueous methanol.

Although the modified process requires two steps in order to obtain the imine-type compounds of formula I from the diaminothioacetal compounds obtained in step (vi), as against the single step of the cyclization procedure first described, the two step process can give a better overall yield than the one step process and requires a less complex isolation procedure for the desired product.

In formula I, the compounds according to the invention are shown in the imine form at the N10-C11- and N10'-C11'- positions. In the presence of water, the imine form is converted to the carbinolamine form, that

is



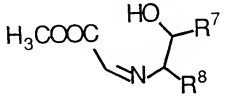
and in the presence of alcohols R^5OH, such as methanol, ethanol and propanol, or the corresponding thiols R^5SH, the imine is converted into the analogous ether or thioether forms, that is

As already indicated, compounds containing such substituents in the 11- and 11'- positions (with the nitrogen in the 10- and 10'- positions in the amine form) are a preferred embodiment of the invention as they are generally more stable than the corresponding N10-C11 imine compounds from which they are derived and since they are convertible to such imine compounds, they are believed to constitute useful pro-drugs.

The imine form is also readily attacked by other nucleophilic reagents, such as ethylamine and thiophenol, or water-soluble sulphur-containing nucleophiles, such as cysteine or glutathione, to form substituents in the 11- position. Such nucleophiles containing polar functional groups can enhance the overall water-solubility of the PBD dimers. Under the appropriate conditions, all of the C11- substituents can be eliminated in order to reform the N10-C11 imine species. To prepare PBD dimers according to the invention in which the C rings contain an oxygen ring atom requires modification of the process described above. Steps (i) to (iv) of the above process are used and the modified process then comprises:

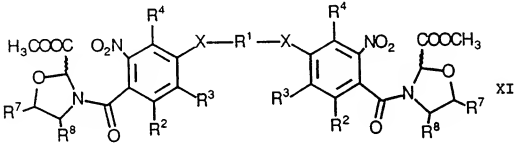
(viii) condensing the dinitro-diacid compound

obtained from step (iv), or the diacid chloride obtainable from said dinitro-diacid, with an imino alcohol of the formula:



X

where R^7 and R^8 have the above-stated meanings, to form a di-N-(2-nitrobenzoyl)oxazolidine ester of the formula:



XI

(ix) reducing the ester groups of the nitrobenzoyl-oxazolidine ester of formula XI to aldehyde groups (-CHO), and

(x) reductively cyclizing the dialdehyde from step (ix) to form a PBD dimer of formula I (in the 10,11-carbinolamine form).

The imino alcohol X is conveniently obtained by condensing methyl glyoxalate with ethanolamine or an appropriately substituted ethanolamine. This condensation may be carried out, for example, in the presence of methylene chloride and molecular sieves at room temperature.

The condensation of step (viii) may be carried out, for example, in the presence of methylene chloride and pyridine with cooling to 0°C.

The reduction of the ester groups to aldehyde groups in step (ix) may be carried out using any of the reagents and conditions conventionally used for this purpose. A preferred procedure is to use di-isobutyl-aluminium hydride as the reducing agent in a toluene reaction medium at a substantially reduced temperature, for example -78°C.

The final reductive cyclization, step (x), may be carried out under mild conditions, for example by hydrogenation in the presence of Pd-C in methanol at room temperature or with stannous chloride ($SnCl_2.2H_2O$) in methanol at reflux temperature.

A particularly preferred compound according to the invention is that in which, referring to formula I, there are methoxy substituents in the 7- and 7'-positions, X is O and R^1 is propylene; this compound is referred to hereinafter as DSB-120. Its preparation is described in the Example below.

The compound DSB-120 was characterised by ^{1}H-NMR, ^{13}C-NMR, infra red, and mass spectrometry. DSB-120 is a stable, pale yellow viscous oil, highly soluble in chloroform, methanol, and ethanol and sparingly soluble in water. It is isolated in the N10- C11 imine form after purification by column chromatography; treatment of the compound in this form with methanol forms the bis(C11S-methyl ether) which is characteristic of the PBD ring system.

An <u>in vitro</u> DNA cross-linking assay by the method described by Hartley et al, <u>Analyt. Biochem.</u>, <u>19</u>, 131-134 (1990) has established that DSB-120 completely cross-links pBR322 DNA at a concentration of 1.0 μM after incubation for one hour at 37°C; a significant level of cross-linking can still be observed with concentrations of DSB-120 as low as 0.001 μM (under the same incubation conditions). The DNA cross-linking efficiency of DSB-120 is also supported by thermal

denaturation data. Treatment of calf thymus DNA with DSB-120 at room temperature causes a 10°C increase in helix melting temperature, compared to an untreated control.

The PBD-dimer described by Farmer et al apparently required incubation at 65°C for one hour at a concentration of 250 uM to effect cross-linking of DNA, that is to say a substantially higher concentration of the PBD-dimer and more vigorous conditions (65°C as against 37°C) than are required for DSB-120.

The adduct formed between DNA and DSB-120 is also substantially more stable than that formed by Farmer et al's PBD-dimer. Thus Farmer et al reported electrophoretic evidence showing that their dimer adduct was dissociated, that is the dimer was released from the DNA, after treatment at pH10 for 12 hours at 25°C.

pBR322 DNA was cross-linked with DSB-120 at a concentration of 0.01μM and excess DSB-120 was then removed by precipitating and isolating the cross-linked DNA. The latter was then incubated at 37°C at pH values of 4.0, 7.0, 8.0, 9.0 and 10.0. A cross-linking assay by the Hartley et al procedure referred to above showed that after 18 hours incubation at each of these pH's, no reversal of cross-linking had occurred. In fact, at the lowest pH (4.0), the DNA control started to show signs of degradation, whereas the cross-linked DNA appeared to remain static. The stability of the cross-linked DNA after 18 hours incubation at pH 10 is directly contrary to the dissociation of the Farmer et al adduct after incubation at pH 10 for 12 hours at 25°C.

Preliminary NMR data suggest that DSB-120 cross-links double-stranded DNA by binding across two guanine residues on opposite strands with two AT base-pairs in between.

The IC_{50} (inhibitory concentration, 50%) of DSB-120 has been evaluated in vitro in L1210, PC6 and

CH1 cell lines using a standard screening procedure. The table below indicates that with one exception (mitoxantrone/CH1), DSB-120 is significantly more cytotoxic across all cell lines than a number of known antitumour agents. It is also noteworthy that the IC_{50} values for DSB-120 in all cell lines are significantly lower than those for the parent PBD (DC-81) from which it is derived.

	IC_{50} μM		
Compound	L1210	ADJ/PC6	CH1
DSB-120	0.01	0.0005	0.003
DC-81	0.38	0.33	0.1
Cisplatin	0.5	0.02	0.1
Adriamycin	0.04	0.04	0.002
Mitoxantrone	N/A	N/A	0.0007
Melphalan	3.0	0.02	2.0
Trimelamol	20.0	5.0	15.0

According to a further aspect of the present invention, there is provided a pharmaceutical composition comprising a PBD dimer of formula I above and an inert, physiologically compatible carrier.

The pharmaceutical composition may take the form of an injectable formulation, that is a solution in saline or saline containing a small proportion, say 10%

by weight, of ethanol or another organic solvent; or a formulation for oral administration, such as a tablet, capsule, solution or syrup; or a composition for topical applicaton, such as a gel, cream or ointment.

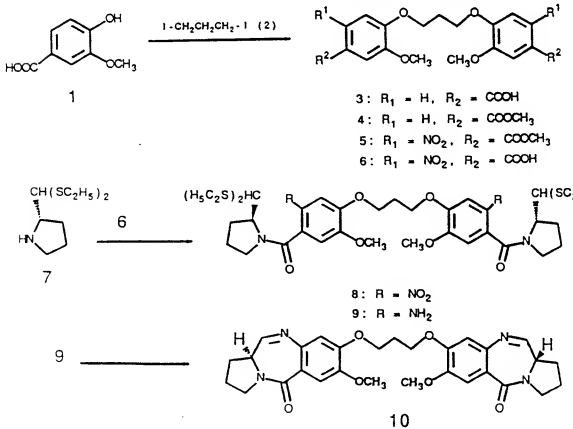
In addition to their physiological activity, the compounds according to the invention are useful experimental reagents for molecular biologists since they can cross-link DNA in a substantially non-reversible manner at a concentration of as low as 0.001 μM. These compounds fluoresce upon binding to DNA and may be used to stain the nuclei of cells, thus being useful in histological-type studies.

In order that the invention may be more fully understood the following examples are given by way of illustration only.

Example 1

Preparation of 1,1'- Propane-1,3-diyldioxybis(11a S)7-methoxy-1,2,3,11a-tetrahydro-5H-pyrrolo[1,2-c] [1,4]benzodiazepin-5-one (DSB-120).

Reference will be made in the following description to the following reaction scheme



(i) 1',3'-Bis(4-carboxy-2-methoxyphenoxy)propane (3)

Vanillic acid (1), (10g, 59.5mmol) was dissolved in tetrahydrofuran (THF) (100ml) and aqueous NaOH (0.5M, 225ml). A solution of 1,3-diiodopropane (2) (8.8g, 29.7mmol) in THF (50ml) was added dropwise with vigorous stirring at 65°C over 4 hours and then refluxed for 48 hours. After cooling, the mixture was washed with hexane (3 x 100ml) and the THF removed by evaporation in vacuo. The aqueous residue was acidified to pH 1 with HCl (36%) and the resultant precipitate was filtered and recrystallised from acetic acid to afford (3) as an off-white amorphous solid; yield: 7.4g (66%), m.p. 238-240°C. MS (EI): m/z (relative intensity) = 376 (M^+, 28), 360 (3), 249 (2), 209 (45), 165 (29), 153 (16), 151 (19), 137 (19), 121 (7), 78 (15), 44 (100). IR (KBr): ν = 3600-2000, 1680 (C=O), 1600 (C=C), 1515, 1465, 1430, 1345, 1310, 1270, 1225, 1180, 1140, 1115, 1030, 990, 970, 950, 925, 875, 850, 825, 765, 725, 645 cm^{-1}. ^1H NMR (DMSO-d_6) δ = 2.23 (t, 2H,J=6Hz), 3.80 (s, 6H), 4.20 (t, 4H, J=6Hz), 7.09 (d, $2H_{arom}$, J=8.4Hz), 7.45 (d, $2H_{arom}$, J=1.8Hz) 7.54 (d, $2H_{arom}$, J=8.4Hz, 1.8Hz fine coupling), 12.76 (bs, $2H_{acid}$). ^{13}C NMR ($CDCl_3$): δ=28.4, 55.4, 64.8, 111.9, 112.0, 122.9, 123.0, 148.3, 151.6, 167.0.

(ii) 1',3'-Bis(2-methoxy-4-methoxycarbonylphenoxy) propane (4)

Oxalyl chloride (3.1g, 24.4mmol, 2.4 equiv.) was added to a stirred suspension of (3) (3.76g, 10mmol) and dimethyl formamide (DMF) (2 drops) in dry THF (25ml), and the stirring was continued for 6 hours. The THF was removed by evaporation in vacuo and the resultant solid quenched with dry methanol (60ml). After the addition was complete, the mixture was stirred for a further 2 hours followed by removal of the methanol. The resultant residue was triturated with

water, and (4) was collected by filtration as a brown amorphous solid; yield: 3.8g (94%). m.p. 153-155°C. $C_{21}H_{24}O_8$ calc. 404.1472, found 404.1457: MS (EI):m/z (relatively intensity) = 404 (M^+, 92), 373 (19), 256 (3), 223 (100), 195 (49), 191 (58), 171 (18), 164 (39), 151 (51), 135 (12), 119 (20), 107 (11), 85 (61), 83 (90), 59 (23), 43 (24). IR (KBr): ν= 2940, 1710 (C=O), 1600 (C=C), 1510, 1460, 1435, 1410, 1340, 1290, 1270, 1215, 1180, 1130, 1100, 1060, 1025, 975, 870, 760, 720 cm^{-1}. ^1H NMR ($CDCl_3$): δ = 2.39-2.43 (m, 2H), 3.89 (s, 6H), 3.90 (s, 6H), 4.30 (t, 4H, J=6.1Hz), 6.93 (d, 2H, J=8.5Hz), 7.54 (d, 2H, J=2.0Hz), 7.64 (dd, 2H, J=8.5Hz, fine coupling = 2.0Hz). ^{13}C NMR ($CDCl_3$): δ = 28.9. 52.0, 56.0, 65.4, 111.7, 112.3, 122.8, 123.5, 148.9, 152.2, 166.9.

(iii) 1',3'Bis(2-methoxy-4-methoxycarbonyl-5-nitrophenoxy)propane (5)

A freshly prepared mixture of $SnCl_4$ (4.6g, 17.7mmol) and fuming nitric acid (1.42g, 22.5mmol) in CH_2Cl_2 (5ml) was added dropwise over 5 minutes with stirring to a solution of the methyl ester (4; 2.85g, 7.1mmol) in CH_2Cl_2 (60ml) at -25°C (dry ice/CCl_4). The mixture was maintained at the same temperature for a further 10 minutes, quenched with water (150ml), and then allowed to return to room temperature. The organic layer was separated and the aqueous phase extracted with ethyl acetate (2 x 100ml). The combined organic phase was dried (Na_2SO_4) and evaporated in vacuo to afford the nitro dimer methyl ester (5) as a brown gum (3.0g) which was recrystallised from ethyl acetate/hexane to afford yellow prisms; 2.97g (85%); m.p. 165-168°C. $C_{21}H_{22}N_2O_{12}$ Calc. 494.1173, found 494.1170. MS (EI):m/z (relative intensity) = 494 (M^+, 94), 463 (14), 449 (8), 404 (3), 285 (3), 268 (28), 267 (29), 240 (32), 236 (68), 222 (94), 207 (6), 194 (45), 164 (19), 151 (15),

122 (14), 109 (9), 86 (47), 84 (73), 75 (12), 59 (29), 49 (100), 41 (73). IR (KBr): ν = 2940, 1710 (C=O), 1600 (C=C), 1575, 1520 (NO_2), 1420, 1355 (NO_2), 1280, 1250, 1210, 1175, 1140, 1045, 990, 960, 870, 830, 750 cm^{-1}. ^1H NMR ($CDCl_3$); δ = 2.43 (m, 2H), 3.90 (s, 6H), 3.95 (s, 6H), 4.32 (t, 4H, J=6.0Hz), 7.06 (s, 2H), 7.49 (s, 2H). ^{13}C NMR ($CDCl_3$): δ = 28.6, 53.3, 56.6, 65.6, 108.1, 110.9, 121.9, 149.5, 152.8, 167.0.

(iv) 1',3'-Bis(4-carboxy-2-methoxy-5-nitro-phenoxy)propane (6)

To a solution of (5) (0.7g, 1.4mmol) in THF (20ml) was added aq. NaOH (1M, 10ml) and the reaction mixture stirred at room temperature for 6 hours until TLC (EtOAc/hexane, 1:1) indicated that reaction was complete. The THF is removed by evaporation in vacuo, and the resultant residue acidified to pH 1 with HCl (35%). The precipitate is collected by filtration and dried to afford (6) as a yellow solid; yield: 0.62g (94%). m.p. 243-246°C. MS (EI): m/z (relative intensity) = 467 ($MH^{+\cdot}$,1), 450 (1), 436 (3), 423 (8), 378 (4), 268 (1), 255 (4), 236 (4), 210 (7), 194 (2), 182 (7), 164 (14), 153 (2), 123 (3), 91 (6), 77 (3), 55 (5), 44 (100). IR (KBr): ν = 3620-2280, 1700 (C=O), 1595 (C=C), 1570, 1515 (NO_2), 1460, 1415, 1350 (NO_2) 1270, 1210, 1180, 1135, 1045, 930, 880, 810, 750, 730, 645cm^{-1}. ^1H-NMR(DMSO-d_6): δ = 2.25 (t, 2H, J=5.9Hz), 3.90 (s, 6H), 4.27 (t, 4H, J=5.9Hz), 7.29 (s, 2H$_{aromatic}$), 7.62 (s, 2H$_{aromatic}$), 13.6 (bs, 2H$_{acidic}$). ^{13}C-NMR (DMSO-d_6): δ = 28.0, 56.3, 65.7, 108.0, 111.2, 121.1, 141.3, 149.1, 151.7, 165.9.

(v) 1,1'-Propane-1,3-diyldioxybis(2-nitro-5-methoxy-1,4-phenylene)bis(carbonyl)bis[pyrrolidine-2-carbaldehyde di(ethylthio)acetal] (8)

Dimethyl formamide (2 drops) was added to a

stirred suspension of (6) (0.5g, 1.07mmol) and oxalyl chloride (0.34g, 2.69mmol, 2.5 equiv.) in dry THF (15ml) and the stirring was continued for 4 hours. The THF was removed by evaporation in vacuo, and the resultant yellow solid was dissolved in dry THF (10ml) and added dropwise over a period of 25 min to a vigorously stirred suspension of (2S)-pyrrolidine-2-carbaldehyde diethyl thioacetal (0.55g, 2.68mmol, 2.5 equiv; prepared in five steps from (S)-proline by the method of Langley and Thurston, J. Org. Chem., 52, 91 (1987)), triethylamine (0.46g. 4.5mmol, 4.2 equiv.) and ice/water (0.6ml) cooled in an ice bath. After addition was complete, the mixture was warmed to room temperature and stirred for a further 1.5 hours. After removal of the THF by evaporation in vacuo, the residue was diluted with water (2 x 50ml) and extracted with ethyl acetate (3 x 25ml). The combined organic phase was washed with sat. $NaHCO_3$ solution (2 X 25ml), dilute HCl (2M, 2 X 25ml) and brine (3 X 25ml), dried (anhyd. $MgSO_4$) and the solvent then removed by evaporation in vacuo to afford a dark red oil which was purified by flash chromatography (ethyl acetate/hexane, 1:1; TLC: ethyl acetate/hexane, 3:2) to afford the diamide (8) as a pale yellow oil. Yield: 0.53g (58%). IR (neat):ν2930, 1625 (C=O), 1570 (C=C), 1510 (NO_2), 1445, 1420, 1370, 1330, (NO_2), 1270, 1215, 1050, 750cm^{-1}. 1H-NMR ($CDCl_3$) δ = 1.25-1.38 (m,14H), 1.76-2.47 (m, 8H), 2.67-2.87 (m,8H), 3.21-3.30 (m, 4H), 3.95 (s, 6H) 4.30-4.35 (m, 4H), 4.67-4.74 (m, 2H), 4.87 (d, 2H, J=3.9Hz), 6.83 (s,$2H_{aromatic}$),7.72 (s, $2H_{aromatic}$). ^{13}CNMR δ = ($CDCl_3$) 15.0, 24.6, 26.3, 26.6, 27.2, 50.2, 52.8, 56.5, 61.1, 65.6, 108.4, 109.3, 128.5, 137.2, 148.2, 154.5, 166.5.

(vi) 1,1'-Propane-1,3-diyldioxybis(2-amino-5-methoxy-1,4-phenylene)bis(carbonyl)bis(pyrrolidine-2-carbaldehyde-di(ethylthio)acetal] (9)

A solution of the dinitro thioacetal (8) (0.5g, 0.59mmol) and $SnCl_2.2H_2O$ (1.37g, 6.0mmol) in methanol (15ml) was refluxed for 40 min until TLC (ethyl acetate/hexane, 4:1) indicated that the reaction was complete. The solvent was removed by evaporation *in vacuo* and the residue cooled to 0°C and neutralised with sat. $NaHCO_3$. The resulting solid was then triturated with ethyl acetate (2x50ml) and the mixture was stirred at room temperature for 1 hour. The suspension was filtered through a short bed of celite, which was rinsed with more ethyl acetate (2x50ml). The combined filtrate was separated and the organic phase was evaporated *in vacuo* to afford the diamino thioacetal (9) as a light yellow foam. Further purification by flash chromatography (ethyl acetate, 100%) afforded a yellow oil contaminated with a small amount of solid stannic salts, which was used directly for the next step. Yield: 0.3g (65%). IR (neat) ν = 3425 and 3315 (NH_2), 2930, 1620, 1590, 1505, 1460, 1455, 1400, 1260, 1165, 1030, 750 cm^{-1}.

(vii) 8,8'-[1,3-Propanediylbis(oxy)]bis[7-methoxy-1,2,3,11a(S)-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5-one] (10,DSB-120)

A solution of the diamino thioacetal (9) (0.3g, 0.39mmol), $HgCl_2$ (0.52g, 1.92mmol, 5 equiv.), and $CaCO_3$ (0.19g, 1.92mmol, 5 equiv.) in CH_3CN/H_2O (4:1, 15ml) was slowly stirred at room temperature for 2.5 hours until TLC ($MeOH/CHCl_3$, 1:4) indicated complete loss of starting material. After removal of the CH_3CN/H_2O by evaporation *in vacuo* at room temperature, MeOH (15ml) was added to the residue and the suspension filtered and/or directly loaded onto a chromatography column (silica gel, 400 mesh). The column was first eluted with ethyl acetate (HPLC grade) to remove the

$HgCl_2$ salts, and then with $CHCl_3$ (HPLC grade, 200ml). Gradient elution with MeOH/$CHCl_3$ (3-5% MeOH) afforded (10) as a light yellow oil. Yield 0.17g (83%). ^1H NMR ($CDCl_3$); δ = 2.01- 2.17 (m,2H), 2.28-2.45 (m, 8H), 3.50-3.87 (m, 6H), 3.92 (s, 6H), 4.22-4.33 (m, 4H), 6.85 (s, 2H), 7.5 (s, 2H), 7.66 (d, 2H, J=4.4Hz). ^{13}C NMR ($CDCl_3$): δ = 24.2, 28.8, 29.6, 46.7, 53.7, 56.1, 65.4, 110.7, 111.6, 120.3, 140.6, 147.8, 150.6, 162.4, 164.6. MS (FAB): m/z=533 (M^++1). Conversion to the C11(S) methyl ether form was effected by dissolving in CH_3OH: ^1H-NMR (CD_3OD) : δ = 2.0-2.3 (m, 10H,) 3.3 (s, 6H), 3.47-3.72 (m, 6H), 3.8 (s, 6H), 4.22-4.23 (m, 4H), 4.40 (d, 2H, J=9.0Hz), 6.61 (s, 2H), 7.15 (s, 2H), 7.90 (s,2H). A small amount of the C11(R) isomer was evidence from signals at 4.58 (s, H11), 6.35 (s) and 7.34 (s).$[\alpha]_D^{23}$=+330°(c=0.6,$CHCl_3$).

Example 2

DNA cross-linking efficiency was evaluated using the method of Hartley et al (Analyt. Biochem., 19, 131-134 (1990)). The reaction of DSB-120 with double-stranded, end-labeled, pBR322 DNA in a concentration range of 0.001-100μM was allowed to proceed in a triethanolamine/EDTA (25mM:1mM, pH7.2) buffer at 37°C. After 1h incubation, the reaction was stopped by the addition of an equal volume of sodium acetate/EDTA (0.6M:20mM) buffer and tRNA (100μg/ml). The DNA was precipitated by the addition of 95% ethanol (3 vols), and the resulting pellet, after centrifugation and removal of the supernatant, was dried and lyophilized. Samples were redissolved in a strand separation buffer (10μM of 30% DMSO/1ml EDTA/0.04% bromophenol blue/0.04% xylene cyanol), heated to 90°C for two minutes to ensure denaturation, and then chilled in ice water immediately prior to gel electrophoresis.

Controls consisting of undenatured samples were dissolved directly in loading buffer (10μg of 6% sucrose/0.04% bromophenol blue) prior to loading.

Electrophoresis was performed on 20cm

submerged agarose gels (0.8%) at 40V for 16h. The gel and running buffer consisted of 40mM Tris, 20mM acetic acid and 2mM EDTA (pH 8.1). Autoradiography relative band intensities were determined by microdensitometry using a LKB Ultrascan-XL laser densitometer.

These experiments showed that DSB-120 completely cross-links the experimental DNA at a concentration of 1.0 μM and that significant cross-linking is observed down to a concentration of 0.001 μM.

The stability of the adduct to low pH and high temperature was also investigated. A sample of end-labelled linearised pBR322 DNA previously treated with DSB-120 was centrifuged to precipitate cross-linked DNA, and then unreacted DSB-120 was removed in the supernatant. The cross-linked DNA was then incubated for 15 minutes in buffers at pH values 4.0, 7.0, 8.0, 9.0 and 10.0. Following the procedure of the cross-linking assay described above, no reversal of cross-linking was observed at these pH values. In addition, heating the adducts at 90°C for two minutes which is part of the assay protocol, also failed to reverse cross-linking.

Examples 3-5

Ex. 3: 8,8'-[1,4-Butanediylbis(oxy)]bis[7-methoxy-1,2,3,11a(S)-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5-one]; (AT-111)

Ex. 4: 8,8'-[1,5-Pentanediylbis(oxy)]bis[7-methoxy-1,2,3,11a(S)-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5-one]; (AT-286)

Ex. 5: 8,8'-[1,6-Hexanediylbis(oxy)]bis[7-methoxy-1,2,3,11a(S)-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5-one]; (DSB-131)

The above three compounds of formula I in which X is -O-, R^1 is butylene, pentylene, and hexylene, respectively, and the 7- and 7'- positions are substituted by methoxy groups, were prepared by the method described in Example 1 with the appropriate substitutions of the diiodoalkane reagent (2). Thus in Example 3, 9.2g of 1,4-diiodobutane were used in place of 1,3-diiodopropane; in Example 4, 9.6g of 1,5-diiodopentane; and in Example 5, 10.0g of 1,6-diiodohexane.

The characteristics of these compounds and the yields of the final steps of the synthesis were as follows.

Ex. 3

^{1}H-NMR ($CDCl_3$): δ = 2.02-2.17 (m), 2.29-2.34 (m,7H), 3.50-3.87 (m), 3.93 (s, 6H), 4.12-4.21 (m, 4H), 6.81 (s, 2H), 7.50 (s, 2H), 7.66 (d,2H, J=4.4Hz). ^{13}C-NMR ($CDCl_3$): δ = 24.2, 25.7, 29.6, 46.7, 53.7, 56.1, 65.4, 110.7, 111.6, 120.3, 140.6, 147.8, 150.6, 162.4, 164.6, Yield 65% from 0.52g (0.65mmol).

Ex. 4

^{1}H-NMR($CDCl_3$): δ = 1.61-1.66(m), 1.95-2.04(m), 2.28-2.30 (m), 3.57-3.84(m), 3.93(s), 3.97-4.07(m), 6.80(s,2H), 7.51 (s,2H), 7.64(d, 2H, J=11.9Hz): ^{13}C-NMR($CDCl_3$): δ = 24.2, 22.5, 28.6, 29.7, 46.7, 53.7, 56.2, 68.5, 110.4, 111.5, 120.1, 140.6, 147.8, 150.8, 162.4, 164.7: Yield 60% from 0.53g (0.65mmol). $[\alpha]_D^{23}$=+47.8°(c=0.4, $CHCl_3$).

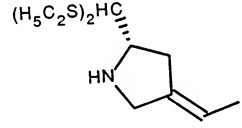
Ex. 5

^{1}H-NMR($CDCl_3$): δ = 1.54-1.56 (m,4H), 1.89-2.08 (m,8H), 2.11-2.33 (m,4H), 3.47-3.85 (m,6H), 3.89 (s,6H), 4.03-4.09 (m,4H), 6.80 (s,2H), 7.50(s,2H), 7.66(d,2H, J=4.4Hz): ^{13} C-NMR($CDCl_3$): δ = 24.2, 25.7, 28.8,29.6, 46.6, 53.7, 56.2, 68.8, 110.4, 111.5, 120.1, 140.6, 147.8, 150.8, 162.4, 164.7: Yield 82% from 0.53(0.65mmol). $[\alpha]_D^{23}$=+470.5° (c=0.18,$CHCl_3$).

Example 6

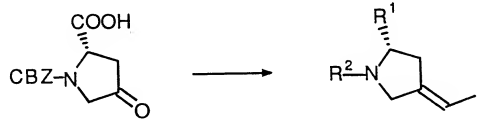
8,8'-[1,3-Propanediylbis(oxy)]bis[(E,Z)-2-ethylidene-7-methoxy-1,2,3,11a(S)-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5-one];(17)

This compound of formula I in which X is -O-, R^1 is propylene, the 2- and 2'- positions are substituted by ethylidene groups, and the 7- and 7'-positions are substituted by methoxy groups, was prepared by a method generally similar to the method of Example 1 subject to the replacement of the reactant (2S)-pyrrolidine-2-carbaldehyde diethyl thioacetal (compound 7 of Example 1) by the compound, (2S)-(E,Z)-4-ethylidenepyrrolidine-2-carboxaldehyde diethyl thioacetal (16), of the formula:



16

Compound 16 was prepared from (2S)-N-(benzoxycarbonyl)-4-oxo-pyrrolidine-2-carboxylic acid (11) by the following reaction scheme:



11

12: R'= COOH, R^2 = CBZ
13: R'= $COOCH_3$, R^2 = CBZ
14: R' = CHO, R^2 = CBZ
15: R' = $CH(SEt_2)_2$, R^2 = CBZ
16: R' = $CH(SEt_2)_2$, R^2 = H

(2S)-(E,Z)-N-(Benzoxycarbonyl-4-ethylidenepyrrolidine-2-carboxylic acid (12)

Potasium t-butoxide (18.6g, 152mmol, 4.0 equiv) and triethylphosphonium bromide (28.2g, 76mmol, 2.0 equiv) were added to a stirred solution of (2S)-N-(benzoxycarbonyl)-4-oxo-pyrrolidine-2-carboxylic acid (11;10.0g, 38mmol) in freshly distilled and dried (sodium) THF (300ml). The mixture was stirred at room temperature under dry nitrogen for 30 minutes and then refluxed for 6 hours. The resulting mixture was filtered and the filtrate evaporated in vacuo to afford a crude brown oil. Further purification by flash chromatography (1:1, ethyl acetate/hexane) gave 6.79g (56%) of a mixture of E and Z isomers as a golden yellow oil.
^{1}H-NMR ($CDCl_3$): δ=1.60-1.63 (m,3H,=CHC$\underline{H}_3$), 2.66-3.00 (m,2H,-N-CH_2-CO-C$\underline{H}_2$-), 3.98-4.20 (m,2H,-N-C$\underline{H}_2$-CO-CH_2-), 4.50-4.58 (m,1H , -C$\underline{H}$-COOH), 5.09-5.28 (m,2H, -C$\underline{H}_2$-O-CO-), 5.41 (bs, 1H,=C$\underline{H}$-CH_3), 7.25-7.50 (m, 5H_{arom}), 7.60 (bs, 1H, -COO$\underline{H}$); ^{13}C-NMR ($CDCl_3$) [E and Z isomers] (rotomers): δ = (14.4, 14.7),[(31.4,32.5)(35.4, 36.5)], [(47.8,48.3)(50.8,51.1)][(58.3,58.5,58.7,59.0)], (67.3,67.5),(118.0,118.4),(127.9,128.4,128.5),(132.8,132.9),133.7,(136.3, 136.4), (154.5,155.6), (176.7,177.6); IR (neat film) ν=2500-3600, 1650-1750(C-O),1585, 1500, 1420,1350,1295,1230,1120,1050,1025,950,910,750,700;MS (ACE,isobutane): m/z(relative intensity) = 275(M^+,6),230 (8), 201 (4),186(26),140(70),96(15),91(100),65(22); MS(CI,isobutane)276(MH^+),232

Methyl(2S)-(E,Z)-N-(benzoxycarbonyl)-4-ethylidene-pyrrolidine-2-carboxylate(13)

Oxalyl chloride (75g,0.59mol) was added dropwise over 30 minutes to a stirred solution of (2S)-(E,Z)-N-(benzoxy-carbonyl)-4-ethylidenepyrrolidine-2-carboxylic acid (12; 80g,0.27mol)dissolved in dry toluene (400ml) at 0°C (ice/water bath). Four drops of DMF was added and

stirring continued for 12 hours at room temperature. Freshly distilled dry methanol (200mL) was added to the mixture and the reaction stirred for a further 4 hours. The solvent was evaporated in vacuo and the residual oil dissolved in ethyl acetate (100mL), washed with saturated aqueous $NaHCO_3$ (4 x 50mL), brine (100mL), then dried ($MgSO_4$) and the solvent evaporated in vacuo to afford 79g (100%) of 13 as a pale yellow oil; ^{1}H-NMR ($CDCl_3$): δ=1.59-1.63 (m,3H,=CH-$\underline{CH_3}$),2.57-2.68 and 2.83-3.00 (m,2H,-N-CH_2-CO-$\underline{CH_2}$-), 3.72 and 3.73 (s,3H, -COO$\underline{CH_3}$), 4.07-4.12 (m,2H,-N-$\underline{CH_2}$-CO-CH_2), 4.46-4.62 (m, 1H,-$\underline{CH}$-COOH), 5.04-5.11(m,2H,-$\underline{CH_2}$-O-CO-), 5.40 (bs, 1H, =$\underline{CH}$-CH_3), 7.27-7.38 (m,5H_{arom}); ^{13}C-NMR($CDCl_3$) [E and Z isomers] (rotomers): δ=(14.5,14.7),[(31.7,32.6)(35.7, 36.5)],[(47.8,48.4)(50.7,51.1)],52.2,53.6,[(58.5,58.7, 58.9)], 67.1, [(117.7, 117.8, 118.2)], 127.8, 127.9, 128.4, [(133.1, 133.2)(134.0,134.1)], [(136.5,136.6)], [(154.3,154.5)(154.9,155.0)], 172.9; IR (neat film) ν = 2800-3100, 1745 (C=O), 1705 (C=O),1410,1350,1210,1180, 1115, 1025, 775, 700cm^{-1}; MS (EI): m/z (relative intensity) =289 (M^+,2), 244 (8), 230 (87), 186 (20), 154 (15), 91 (100), 85 (12), 65 (35); MS (CI, isobutane); 290 (MH^+)

(2S)-(E,Z)-N-(Benzoxycarbonyl)-4-ethylidenepyrrolidine-2-carboxaldehyde(14)

A solution of $(i\text{-}Bu)_2AlH$ [DIBAL-H] (100mL of a 1M solution in toluene) was added dropwise over a period of 45 minutes to a solution of the methyl ester (13;10g, 34.6mmol) in dry toluene (150mL) maintaining the temperature at -78°C (acetone/dry ice) under a nitrogen atmosphere. The reaction was stirred for a further 30 minutes and then the excess DIBAL-H carefully decomposed by the addition of methanol (100mL) followed by NaOH(0.2M, 100mL). The mixture was allowed to warm to room temperature and the organic layer separated. The aqueous layer was adjusted to pH 11 (NaOH,0.5M) and

extracted with ethyl acetate (3 x 100mL). The ethyl acetate extracts were combined, washed with brine (100mL), dried ($MgSo_4$) and the solvent evaporated in vacuo (<35°C) to afford 5.83g (65%) of 14 as a pale yellow oil. TLC: (ethyl acetate/petroleum ether 1:1) ^{1}H-NMR ($CDCl_3$): δ=1.60-1.63 (m,3H, =CH-C$\underline{H}_3$), 2.40-2.90 (m, 2H, -N-CH_2-C-C$\underline{H}_2$-), 4.08-4.12 (m, 2H,-N-C$\underline{H}_2$-C-CH_2-), 4.15-4.67 (m, 1H, -C$\underline{H}$-COOH), 5.14-5.25 (m, 2H, -C$\underline{H}_2$-O-CO-), 5.42 (bs, 1H,=C$\underline{H}$-CH_3), 7.26-7.36 (m, 5H_{arom}), 9.48 (d,0.35H,J=6.32Hz, Z isomer, 35%, -CH-CHO) and 9.56 (d, 0.65H, J=6.9Hz, E isomer, 65%, -CH-CHO); ^{13}C-NMR ($CDCl_3$) [E and Z isomers] (rotomers): δ = 14.4, [(28.0,29.0)(32.0,33.0)], [(48.0,48.5)(51.0,51.5)], (64.6,64.7), 67.4, [(118.4, 118.9)], 128.0, 128.2, 128.5, [(132.4, 133.4)], 136.3, [(155.0, 156.0)], [(199.3, 199.5)]' IR (neat film)ν=2700-3700, 2900, 1720 (C=O), 1700 (C=O), 1500, 1415, 1350, 1300, 1200, 1180, 1110, 1060, 920, 770, 735, 700cm^{-1}; MS (EI) m/z (relative intensity) =230(50,M^+.-CHO), 186 (37), 154 (9), 91 (100), 65 (21).

(2S)-(E,Z)-N-(Benzocarbonyl)-4-ethylidenepyrrolidine-2-carboxaldehyde diethyl thioacetal (15)

Ethanethiol (7.90g. 127mmol) was added to a stirred solution of the aldehyde (14;15g, 58mmol) in freshly distilled dichloromethane (40mL) under a nitrogen atmosphere. The mixture was stirred for a further 30 minutes followed by the addition of trimethylsilyl chloride (11.65g, 107mmol). The reaction was then stirred under nitrogen for a further 24 hours or until TLC (ethyl acetate/hexane 1:1) indicated that the reaction was complete. The mixture was then carefully neutralised with saturated aqueous $NaHCO_3$ and then extracted with water (3 x 30mL). The combined aqueous phase was back-extracted with chloroform (3 x 20mL) and the combined organic phase dried ($MgSO_4$) and evaporated

in vacuo to afford 16.91g (80%) of the thioacetal (15) as a dark brown oil.

^{1}H-NMR ($CDCl_3$) δ=1.09-1.39 (m, 6H, $CH(SCH_2\underline{CH_3})_2$), 1.56-1.65 (m, 3H, =CH-$\underline{CH_3}$), 2.40-2.60 (m,4H, $CH(S\underline{CH_2}CH_3)_2$), 2.60-2.81 (m, 2H, -N-$CH_2$-C-$\underline{CH_2}$), 4.05-4.20 (m,2H,-N-$\underline{CH_2}$-C-$CH_2$), 4.25-4.58 (m, 2H, $\underline{CH}$-$CH(SCH_2CH_3)_2$ and CH-$\underline{CH}(SCH_2CH_3)_2$), 5.02-5.15 (m, 2H, -$\underline{CH_2}$-O-CO), 5.41 (bs, 1H,=$\underline{CH}$-$CH_3$), 7.12-7.41 (m, 5$H_{arom}$); IR (neat film) ν = 3200-3600, 2800-3100, 1700 (C=O), 1450, 1410, 1350, 1300, 1265, 1245, 1205, 1105, 1045, 975, 920, 750, 700, cm^{-1}; MS (EI): m/z (relative intensity) =365 ($M^{+\cdot}$;3), 303 (12), 292 (4), 230 (43), 186 (53), 168 (6), 152 (9), 135 (16), 107 (6), 91 (100), 75 (5), 65 (14); Calculated for $C_{19}H_{27}NO_2S_2$ 365.1483 found 365.1497.

(2S)-(E,Z)-4-Ethylidenepyrrolidine-2-carboxaldehyde diethyl thioacetal (16)

A solution of trimethylsilyl iodide (5.0mL, 35.4mM, 2.4 equiv) in freshly distilled dry dichloromethane (75mL) was added dropwise with stirring to a solution of 15;5.4g, 14.7mM, 1 equiv) and 2,3-dimethylbutene (5.0ml) in dichloromethane (50mL) under a nitrogen atmosphere. The mixture was stirred for 90 minutes or until reaction was complete as indicated by TLC (ethyl acetate/hexane, 1:9). After quenching with methanol (100mL) and evaporation of the solvent at 35°C in vacuo, the resulting oil was dissolved in ether (150mL) and extracted with HCl (0.5M, 3 x 200 mL). The combined aqueous phase, after adjusting to pH8.0 with 2M NaOH,was back-extracted with ether (4 x 150mL) and the combined organic phase dried ($MgSO_4$) and evaporated in vacuo to afford a crude red oil which was purified by column chromatography (silica gel 400 grade, hexane/ethyl acetate, gradient 9:1 to 4:1) to afford 2.10g (65%) of the amine 16 as a dark brown oil.

^{1}H-NMR ($CDCl_3$): δ= 1.16-1.20 (m, 6H, $CH(SCH_2\underline{CH_3})_2$), 1.55

(d, 1H, J-6.8Hz, Z-isomer, 35%, =CH-CH_3), 1.61 (d, 2H, J=6.8Hz, E-isomer, 65% =CH-CH_3), 2.49-2.80 (m, 6H, -N-CH_2-C-CH_2 and CH(SCH_2CH_3)$_2$), 3.24-3.70 (m, 3H, N-CH_2-C-CH_2 and CH-CH(SCH_2CH_3)$_2$), 3.75 (d,0.35H, J=8.2Hz, Z-isomer, 35%, CH-CH(SCH_2CH_3)$_2$) and 3.80 (d,0.65H, J=8.1Hz, E-isomer, 65%, CH-CH(SCH_2CH_3)$_2$), 5.32 (bs, 1H, =CH-CH_3); ^{13}C-NMR ($CDCl_3$) [E and Z isomers] (rotomers): δ=[14.5, 14.8, 14.6], [24.2, 24.4, 25.0], [33.7, 37.5], [47.8, 51.2], [56.5, 56.7], [61.9, 62.3], [115.0, 115.4], [139.1, 139.2]; IR (neat film) ν=2950, 2920, 1575, 1450, 1360, 1175, 1100, 1050, 975, 845, 815, 780, 745, 695, 600cm^{-1};

The process of Example 1 was then followed to give 8,8'-[1,3-propanediylbis(oxy)]bis[(E,Z)-2-ethylidene-7-methoxy-1,2,3,11a(S)-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5-one], (17) as a light yellow oil.

^{1}H-NMR($CDCl_3$):δ=1.67 and 1.73 (s, 6H, C=CH-CH_3, E and Z), 2.28-2.45 (m, 2H), 2.95-3.05 (m, 4H), 3.83-3.88 (m, 4H), 3.92 (s, 6H), 4.20-4.30 (m, 4H), 5.58 (bs, 2H), 6.88 (s, 2H), 7.48 (s, 2H), 7.66 (d, 2H); MS (FAB): m/z =584 ($M^{+\cdot}$), 505, 490, 479, 465, 448, 425, 415, 359, 343, 329, 311, 301 (586 ($M^{+\cdot}$+2) was also observed); $C_{33}H_{36}O_6N_4$ calculated. 584.2635, found 584.2743; Yield 55% from 0.80g.

Example 7

8,8'-[Oxybis(ethane)-2,2'-diylbis(oxy)]bis[7-methoxy-1,2,3,11a(S)-tetrahydro-5H-pyrrolo-[2,1-c][1,4]benzodiazepin-5-one](18)

This compound of Formula 1 in which X is -O-, R^1 is -$(CH_2)_2$-O-$(CH_2)_2$-, and the 7- and 7'- positions are substituted by methoxy groups, was prepared by the method of Example 1, but using the compound 1,1'-

oxybis(2-iodethane), 19, of the formula:

$$I\text{-}CH_2CH_2\text{-}O\text{-}CH_2CH_2\text{-}I \qquad 19$$

in the first step in place of diiodopropane.

Compound (19) was prepared from 1,1'-oxybis[2-chloroethane] as follows:

1,1'-Oxybis[2-iodoethane] (19)

Sodium iodide (125g,0833mol, 7.1 eqiv) was added to a solution of 1,1'oxybis[2-chloroethane] (16.65g, 0.11mol) in dry acetone (300ml). The solution was then heated under reflux for 40 hours, cooled and the sodium salt filtered. The acetone was evaporated in vacuo. To the residue was added diethyl ether (300ml) and the organic layer washed with sat.aq. solution of sodium thiosulfate (3 x 50ml). The organic layer was dried (Na_2SO_4) and evaporated in vacuo to afford a yellow oil, yield 29.72g (95%). MS (EI): m/z (relative intensity) = 326 (M^+,42), 277 (2), 199 (26), 155 (100), 127 (11); IR (KBr): = 2960, 2880, 1465, 1435, 1420, 1360, 1310, 1270, 1190, 1165, 1110, 1035, 980, 775 cm^{-1}. ^1H NMR ($CDCl_3$):δ = 3.26 (t, 4H, J=6.96 Hz), 3.77 (t, 4H, J=6.96 Hz).

The procedure of Example 1 was then followed to give compound (18) above as a light yellow oil. MS (FAB): m/z (relative intensity) =563, (M^++1, 100).^1H-NMR ($CDCl_3$) : δ=2.05-2.17 (m, 12H), 2.28-2.36 (m, 6H), 3.49-3.89 (m, 6H), 3.92 (s, 6H), 4.01 (t, 4H), 4.18-4.30 (m, 6H), 6.83 (s, $2H_{arom}$), 7.50 (s,$2H_{arom}$), 7.66 (d, 2H, J=4.4Hz). ^{13}C-NMR ($CDCl_3$): δ=24.16, 29.55, 46.70, 53.71, 56.12, 68.38, 69.62, 110.78, 111.62, 120.50, 140.47, 147.82, 150.59, 162.50, 164.61.

Example 8

8,8'-[1,3-Propanediylbis(oxy)]bis[2(S),11(R,S),11a(S)-7,11-dimethoxy-1,2,3,10,11,11a-hexahydro-2-hydroxy-5H-pyrrolo[2,1-c][1,4]-benzodiazepin-5-one] (26)

This compound of formula I in which X is -O-,

R^1 is propylene, the 2- and 2'- positions are substituted with hydroxyl groups and the 7-, 7'-, 11-, and 11'- positions (with the 10,11- and 10', 11'- positions in the carbinolamine form) are substituted with methoxy groups, was prepared by a process analogous to that of Example 1 as follows.

Methyl(2S)-N-(Benzoxycarbonyl)-(4S)-hydroxypyrrolidine-carboxylate (20)

DMF (2 drops) was added to a suspension of the Cbz-acid (10.6g, 40mmol) and oxalyl chloride (7.6g, 59.9mmol, 5.2ml) in anhydrous THF (40ml), and the mixture stirred at room temperature for 5h until TLC (EtOAc/hexane, 1:1) indicated complete reaction. The reaction mixture was evaporated in vacuo, the residue was then quenched with methanol (2 X 25ml) and the mixture stirred for a further 1h. After evaporation of the solvent, the resultant residue was dissolved in EtOAc(2 X 50ml). The solution was washed with aq. $NaHCO_3$ (3 X 50ml), brine (2 X 25ml), and then dried ($MgSO_4$) prior to evaporation in vacuo to afford 10.1g (90%) of pure 20 as a yellow oil. ^{1}H-NMR ($CDCl_3$): δ 2.01-2.12 (m, 1H), 2.24-2.39 (m, 2H), 3.54 (s, 3H), 3.56-3.66 (m, 2H), 4.46-4.54 (m, 2H), 5.02-5.21 (m, 2H), 7.26-7.36 (m, 5H). ^{13}C-NMR ($CDCl_3$, rotamers in brackets): δ 38.4 (39.2), 52.14 (52.4), 54.6 (55.2), 57.7 (57.9), 67.3, 69.3 (70.1), 127.8, 127.9, 128.0, 128.4, 128.5, 136.2 (136.4), 154.6 (155.1), 173.1 (173.2). IR (Neat): ν 3600-3150, 2950-2800, 1745, 1680, 1420, 1350, 1200, 1160, 1120, 1080cm^{-1}. MS (m/z) (EI, relative intensity): 279 (64), 220 (77), 176 (79), 158 (9), 144 (22), 110 (16), 92 (57), 91 (100), 65 (36). HRMS Calcd.for 279.1107 ($C_{11}H_{17}NO_5$), found 279.1106. $[\alpha]_D^{20}$ = -75.8° (c=2.6,$CHCl_3$).

(2S)-N-(Benzoxycarbonyl)-4(S)-hydroxypyrrolidine-2-carboxaldehyde (21)

The Cbz-ester (20;3.51g, 12.6mmol) was dissolved in anhydrous toluene (80ml) and diisobutylaluminum hydride

solution (4.5g, 31.5mmol, 21ml of a 1.5M solution in toluene) added dropwise over a period of 30min at -78°C (liq.N_2/acetone) under a N_2 atmosphere. The mixture was maintained at the same temperature for a further 50min until TLC (EtOAc/hexane, 1:1) indicated complete loss of starting material. The mixture was quenched with methanol (20ml) followed by HCl (1M, 35 ml) and allowed to warm to room temperature. The organic layer was separated and the aqueous phase extracted with EtOAc (4 X 50ml). The combined organic phase was back washed with water (2 X 125ml), brine (2 x 75ml) dried ($MgSO_4$) and the solvent removed in vacuo to afford a yellow oil, which was purified by chromatography (EtOAc/hexane, 6:4) to afford 1.9g (67%) of the corresponding aldehyde 21 as a light yellow oil. ^{1}H-NMR ($CDCl_3$): δ 1.91-2.27 (m, 2H), 2.60 (bs, 1H), 3.52-3.68 (m, 2H), 4.21-4.55 (m, 2H), 5.05-5.20 (m, 2H), 7.29-7.40 (m, 5H), 9.45 and 9.55 (d, 1H, rotomers). ^{13}C-NMR ($CDCl_3$, rotomers in brackets): δ 35.2 (36.0), 55.0 (55.7), 63.5 (63.6), 67.6, 69.1 (70.0), 127.8, 128.0, 128.1, 128.2, 128.5, 135.9 (136.1), 154.9, 199.3 (199.5). IR (Neat); ν 3600-3150, 3030-2800, 1730, 1685, 1415, 1150, 1110, 1050, 990, 750cm^{-1}.

(2S)-N-(Benzoxycarbonyl)-4(S)-hydroxypyrrolidine-2-carboxaldehyde diethyl thioacetal (22)

Ethanethiol (1.1g, 17.9mmol), was added to a stirred solution of the Cbz-aldehyde (21;1.8g, 7.15mmol) in dry $CHCl_3$ (20ml) under a N_2 atmosphere. The mixture was stirred for 40min, followed by the addition of trimethylsilyl chloride (1.93g, 17.8mmol), after a further 12h of stirring under N_2, the reaction mixture was carefully neutralized with saturated $NaHCO_3$ solution and then extracted with water (3 X 20ml). The combined aqueous phase was back-extracted with $CHCl_3$ (30ml) and the combined organic phase dried ($MgSO_4$) and evaporated in vacuo to afford the diethyl thioacetal 22, which was purified by flash chromatography (EtOAc/hexane, 1:1),

(2.21g, 87%). ^{1}H-NMR ($CDCl_3$): δ 1.06-1.34 (m, 6H), 2.15-2.75 (m, 6H), 3.51-3.60 (m, 1H), 3.68-3.81 (m, 1H), 4.45-4.60 (m, 2H), 4.70 and 4.32 (d, 1H, J=3.3Hz, rotamers), 5.01-5.38 (m, 2H), 7.27-7.40 (m, 5H). ^{13}C-NMR ($CDCl_3$): δ 14.5, 14.8, 26.4, 26.9, 36.1 (36.6), 52.9 (53.8), 56.0 (56.5), 60.4 (60.0), 66.7 (67.4), 69.9 (69.5), 127.7, 127.9, 128.3, 128.4, 128.5, 136.6 (136.0), 155.1. MS (m/z relative intensity): (EI) 355 (7), 337 (12), 308 (3), 220 (74), 176 (76), 135 (20), 91 (100), 65 (6). HRMS Calcd. for 355.1276 ($C_{17}H_{25}O_3NS_2$), found 355.1300.

(2S)-4(S)-Hydroxypyrrolidine-2-carboxaldehyde diethyl thioacetal (23)

A solution of Cbz-diethyl thioacetal (22;2.5g, 7.0mmol) in dry dichloromethane (15ml) was added dropwise over 5min to a stirred solution of trimethylsilyl iodide (2.8g, 14.0mmol) in CH_2Cl_2 (15ml) and the mixture stirred for 40min under N_2 atmosphere or until reaction was complete as indicated by TLC (EtOAc/hexane, 3:7). After quenching with methanol (15ml) and evaporation at 35°C in vacuo, the resulting residue was washed with hexane (2 x 100ml), purified by chromatography ($CH_3OH/CHCl_3$, 1:4) to afford 23 (1.25g, 80%) as a dark red low melting solid. ^{1}H-NMR ($CDCl_3$): δ 1.25-1.32 (m, 6H), 1.92-2.02 (m, 1H), 2.21-2.28 (m, 1H), 2.62-2.85 (m, 4H), 3.30-3.58 (m, 2H), 3.93-4.02 (m, 1H), 4.08 (d, 1H, J=8.0Hz), 4.55 (bs, 1H), 5.55 (bs, 2H). ^{13}C-NMR ($CDCl_3$): δ 14.4, 14.5, 24.5, 25.1, 39.2, 54.4, 54.5, 61.2, 70.9. IR (Neat): ν 3500-3060, 2960-2870, 1250-1150, 1020 cm^{-1}. MS (m/z, relative intensity) (CI, isobutane): 222 (100), 202 (3), 185 (3), 160 (37), 86 (11). HRMS Calcd. 221.0908 ($C_9H_{19}ONS_2$), found 221.0957.

1,1'-Propane-1,3-diyldioxybis(2-nitro-5-methoxy-1,4-phenylene)-bis(carbonyl)bis[4(S)-hydroxy-2(S)-pyrrolidine-2-carboxaldehyde diethylthio acetal] (24)

Dimethyl formamide (2 drops) was added to a stirred

suspension of the dimer acid (0.46g, 1.0mmol; see step (iv) of Example 1) and oxalyl chloride (0.318g, 2.5mmol, 2.5 equiv.) in dry THF (20ml), and stirring continued for 4 hours. After evaporation of the THF in vacuo, the resultant yellow solid was dissolved in dry THF (10ml) and added dropwise over a period of 30 minutes to a vigorously stirred suspension of (2S)-4(S)-hydroxy-pyrrolidine-2-carboxaldehyde diethyl thioacetal (23;0.55g, 2.5mmol, 2.5 equiv.), Et_3N (0.42g. 4.2mmol, 4.2 equiv.) and ice/water (0.6ml) cooled in an ice bath. After addition was complete, the mixture was warmed to room temperature and stirred for a further 1.5 hours. After removal of the THF by evaporation in vacuo, the residue was diluted with water (2 x 40ml) and extracted with ethyl acetate (4 X 25 ml). The aqueous phase was adjusted to pH3 with conc. HCl and extracted with ethyl acetate (2 X 25ml). The combined organic phase was washed with water (3 X 25ml) and brine (3 X 25ml), dried (anhyd. $MgSO_4$) and the solvent removed by evaporation in vacuo to afford a dark red oil which was purified by flash chromatography (ethyl acetate/hexane, 1:1; TLC: ethyl acetate/hexane, 3:2) to afford the bis(amide) 24 as a pale yellow oil (0.64g, 73% yield). ^{1}H-NMR ($CDCl_3$): δ 1.30-1.38 (m, 12H), 2.20-2.87 (m, 16H), 3.05-3.16 (m, 2H), 3.42-3.48 (m, 2H), 3.95 (s, 6H), 4.30-4.45 (m, 6H), 4.83-4.89 (m, 4H), 6.82 (s, 2H), 7.64 (s, 2H). ^{13}C-NMR ($CDCl_3$): δ 14.9, 15.1, 26.3, 26.6, 28.9, 36.1, 52.4, 56.6, 58.3, 59.8, 65.1, 69.6, 108.0, 109,5, 127.8, 137.2, 148.2, 154.4, 167.0. IR (Neat): ν 3700-3160, 3040-2800, 1640, 1580, 1520, 1440, 1340, 1280, 1220, 1070, 970, 920, 870, 760 cm^{-1}. MS (FAB): m/z=837 M^++1.

1,1'-Propane-1,3-diyldioxybis(2-amino-5-methoxy-1,4-phenylene)-bis(carbonyl)bis[4(S)-hydroxy-2(S)-pyrrolidine-2-carboxaldehyde diethylthio acetal] 25

A solution of the dinitro thioacetal (24;0.5g, 0.57mmol)

and $SnCl_2.2H_2O$ (1.34g, 6.0mmol) in methanol (25 ml) was refluxed for 40 minutes until TLC (ethyl acetate/hexane, 4:1) indicated that the reaction was complete. The solvent was removed by evaporation in vacuo and the residue cooled to 0°C and treated with sat. $NaHCO_3$. The resulting solid was then triturated with ethyl acetate (3 X 50ml) and the mixture allowed to stir at room temperature for 1 hour. The suspension was filtered through a short bed of celite which was rinsed with ethyl acetate (2 X 50ml). The combined filtrate was evaporated in vacuo to afford the corresponding diamino thioacetal as a light yellow foam. Further purification by flash chromatography (ethyl acetate) afforded a yellow oil 25 (0.35g, 75% yield), which was used directly in the next step. ^{1}H-NMR ($CDCl_3$): δ 1.21-1.39 (m, 12H), 1.62-1.78 (bs, 2H), 2.01-2.18 (m, 2H), 2.21-2.30 (m, 4H), 2.64-2.82 (m, 8H), 3.31-3.41 (m, 2H), 3.63-3.68 (m, 2H), 3.78 (s, 6H), 4.17-4.52 (m, 10H), 4.64-4.66 (m, 2H), 4.85-4.97 (m, 2H), 6.21 (s, 2H), 6.72 (s, 2H). IR (Neat): ν 3710-3140, 3055-2820, 1650, 1595, 1520, 1470, 1460, 1450, 1420, 1340, 1270, 1240, 1180, 1120, 1070, 1030, 980, 870, 840, 760 cm^{-1}.

8,8'-[1,3-Propanediylbis(oxy)]bis[2(S),11(R,S),11a(S)-7,11-dimethoxy-1,2,3,10,11,11a-hexahydro-2-hydroxy-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5-one] 26

A solution of the diamino thioacetal (25;0.4g, 0.5mmol), $HgCl_2$ (0.81g, 3.0mmol, 6.0 equiv.), and $CaCO_3$ (0.3g, 3.0mmol, 6.0 equiv.) in CH_3CN/H_2O (4:1, 15ml) was stirred slowly at room temperature for 2.5 hours until TLC (MeOH/$CHCl_3$ 1:4) indicated a complete loss of starting material. After removal of the CH_3CN/H_2O by evaporation in vacuo at room temperature, the residue was dissolved in MeOH (15ml) and loaded directly onto a chromatography column. The column was first eluted with ethyl acetate (HPLC grade, 5 X 100ml) to remove the

$HgCl_2$ salts, and then with $CHCl_3$ (HPLC grade, 2 X 100ml). Gradient elution with MeOH/$CHCl_3$ (6-10% MeOH) afforded 26 (0.18g, 57%) as a light yellow oil. ^{1}H-NMR (CD_3OD) mixture of C11(R,S)-OCH_3 isomers): δ 2.21-2.46 (m, $-CH_2-\underline{CH_2}-CH_2-$ and 4 X H1), 2.92-3.18 (m, 2 X H3), 3.60-3.98 (m, 2 X C7-OCH_3, 2 X H11a), 4.12-4.39 (m, O-$\underline{CH_2}-CH_2-\underline{CH_2}-O-$, 2 X H2, 2X C_2-OH), 4.41-4.58 (m, 2X H11), 6.39 and 7.38 (s, major; 4H), 7.08 and 7.57 (s, minor, 4H). Small amount of imine was observed as doublet at δ 8.20 for C11 and singlets at δ 6.60 and 7.18 for aromatic protons. ^{13}C-NMR ($CDCl_3$) (mixture of C11 (R,S) diastereomers and small amount of imine): δ 30.1, 39.6, 58.2, 66.2 ($-\underline{CH_2}-$, weaker signals for diastereomers observed at 42.7, 55.0, 56.4, 66.7), 57.2, 59.7, 66.5, 69.4 (CH_3O- and -CH-, additional signal for diastereomer observed at 56.6), 88.6 (C11), 102.9, 106.3, 108.7, 116.3 (C6 and C9), 141.7, 142.9, 146.3, 150.5, 154.3, 156.1, 159.2, 162.1 (C5a, C7, C8, C9a), 169.1 (C=O) other weak signals respectively the imine species were observed at, for example, 110.0, 113.9, 140.0, 146.0, 153.4, 168.9, 171.1 (C11) for the aromatics. MS (FAB): m/z = 565 (M^+-2 X CH_3OH).

Example 9

Experiments have been carried out to determine the concentration of PBD dimers according to the invention required to obtain 50% cross-linking of pBR332 DNA under standard conditions.

In vitro cytotoxicity studies have also been carried out. Compounds were evaluated in the following three cell lines: the L1210 mouse leukaemia (antimetabolite sensitive), the ADJ/PC6 mouse plasmacytoma (alkylating agent sensitive), and the human ovarian CH1 cell line (platinum sensitive). L1210 and ADJ/PC6 were grown as suspension cultures, whereas CH1 was grown as a monolayer. Experimental protocols were as follows:

1210 Leukaemia

Day 0 Cells were set up at 10^4mL in RPMI 1640 medium containing 10% horse serum.

Day 1 Cells were counted to ensure that they were in the logarithmic phase of growth, and then treated with the test compound which was left in contact with the cells for 48h at 37°C.

Day 3 Cells were counted and the percentage inhibition of growth calculated by comparison with controls, which were treated with solvent alone.

ADJ/PC6 Plasmacytoma

Day 0 Cells were set up at 3-5 X 10^4/mL in Dulbecco's medium containing 20% horse serum.

Day I Cells were counted to ensure they were in the logarithmic phase of growth, and then treated with the test compound which was left in contact with the cells for 72H at 37°C.

Day 4 Cells were counted and the percentage inhibition of growth calculated by comparison with controls, which were treated with solvent alone.

CH1 Human Ovarian

Day 0 Cells were set up in flasks (100-200 cells/flask), in Dulbecco's medium containing 10% foetal calf serum, and left overnight to allow the cells to attach.

Day 1 The test compound was added and left in contact with the cells for the duration of the experiment, at 37°C.

Day 9 Colonies of cells were usually visible and were fixed in ethanol (100%), stained with methylene blue and counted.

Compounds were screened over a concentration range starting at 10^{-4}M and decreasing factors of ten until 10^{-11}M. Each compound was dissolved in DMSO so

that the final concentration of DMSO was 0.05% in contact with the cells. Suspension cultures were counted using a Coulter counter and the CH1 colonies counted with a colony counter. The index of activity is expressed as the IC_{50}, that is the dose which inhibits cell growth by 50% compared to the solvent controls.

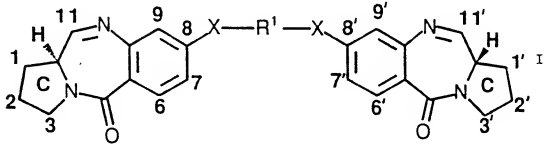
The PBD dimers were also tested for activity against K_{562} human cell line; IC_{50} values were measured using a MTT assay following one hour exposure to test ligand.

The table below summarises the results (for DSB-120 this table repeats certain results previously given):

Compound	50% cross-linking pBR322, μM	IC_{50} μM			
		L1210	ADJ/PC6	CH1	K_{562}
DSB-120	0.055	0.01	0.0005	0.003	0.2
AT 111 (Ex.3)	1.00	1.2	0.35	0.05	2.5
AT-286 (Ex.4)	0.07	0.0045	0.0004	0.00032	0.5
DSB-131 (Ex.5)	0.75	0.34	0.002	0.002	1.0
Ex. 6	0.01	0.00028	0.00008	-	0.01
Ex. 7	0.02	0.22	0.036	-	3.5

CLAIMS:

1. Pyrrolo [2,1-c][1,4] benzodiazepine (PBD) dimers of the formula:



where the PBD tricyclic structures may contain additional substituents in one or more of the 1, 2, 3, 6, 7, 9 and 11- positions and the C rings may optionally contain at least one further hetero ring atom, X is -O-, -S- or -NH-, and R^1 is an alkylene chain containing from 3 to 12 carbon atoms, which chain may, if desired, be interrupted by one or more hetero-atoms and which chain may, if desired, contain one or more carbon-carbon double or triple bonds.

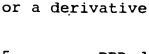
2. PBD dimers according to claim 1, in which the tricyclic structures each contain one or more hydroxyl, alkoxy, ethylidene or 2-acrylamidyl substituents.

3. PBD dimers according to claim 1 or 2, which are substituted in the 2- and 2'- positions by hydroxyl groups or substituents of the formula:

$$-O(CH_2)_n-NH_3^+Cl^-$$

where n is an integer from 1 to 6.

4. PBD dimers according to any of claims 1 to 3, in which the 10,11- positions of the tricyclic structures are in the carbinolamine form:



or a derivative thereof.

5. PBD dimers according to claim 4, in which the 11-position is substituted by an alkoxy or thioalkyl group.

6. PBD dimers according to claim 4, in which the 11- position is substituted by the residue of a nucleophilic reagent.

7. PBD dimers according to claim 6, in which the nucleophilic reagent is ethylamine, thiophenol, or cysteine.

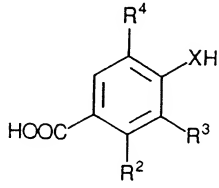
8. PBD dimers according to any of claim 1 to 7, in which R^1 is $-(CH_2)_x-O-(CH_2)_x-$, where x is an integer from 2 to 6, or $-(CH_2)_n-CH=CH-(CH_2)_n-$, where n is an integer from 1 to 5.

9. PBD dimers according to any of claims 1 to 8, in which the C rings contain an -O- ring atom in the 1- and 1'- positions.

10. The PBD dimer according to claim 1, in which there are methoxy substituents in the 7- and 7'- positions, X is O and R^1 is propylene.

11. A method of preparing a PBD dimer of formula I specified in claim 1, which comprises:

(i) condensing a benzoic acid derivative of the formula:

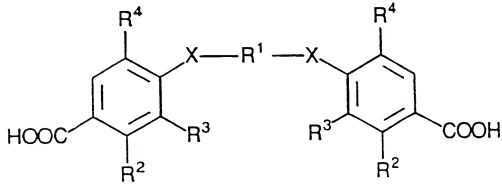


II

where X has the meaning specified in claim 1, R^2 is H or a desired substituent in the 6- or 6'- position of the PBD dimer, R^3 is H or a desired substituent in the 7- or 7'- position of the PBD dimer, and R^4 is H or a desired substituent in the 9- or 9'- position of the PBD dimer, with a dihaloalkane derivative of the formula:

$$X\text{-}R^1\text{-}X \qquad \text{III}$$

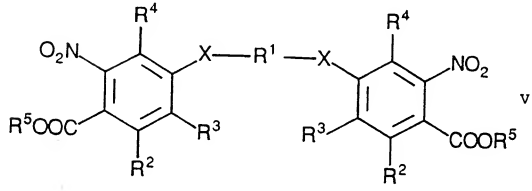
where R^1 has the meaning specified in claim 1 and X is a halogen atom, by adding the dihaloalkane III slowly to a reaction mixture containing the benzoic acid derivative II to form a dimer acid compound of the formula:



IV

(ii) esterifying the dimer acid of formula IV,

(iii) nitrating the diester from step (ii) to obtain a dinitro-diester compound of the formula:

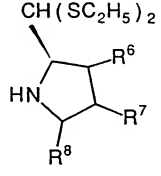


V

where R^5 is an alkyl group,

(iv) hydrolysing the dinitro-diester compound of formula V to remove the esterifying groups and obtain the corresponding dinitro-diacid compound,

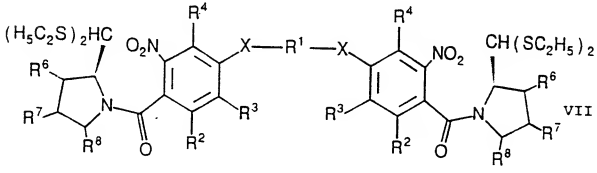
(v) condensing the dinitro-diacid compound from step (iv) with a (2S)-pyrrolidine-2-carbaldehyde diethyl thioacetal of the formula:



VI

where R^6 is H or a desired substituent in the 1- or 1'-position of the PBD dimer, R^7 is H or a desired substituent in the 2- or 2'- position of the PBD dimer,

and $R^{"}$ is H or a desired substituent in the 3- or 3'-position of the PBD dimer, to form a nitrothioacetal derivative of the formula:



VII

(vi) reducing the nitro substituents of the derivative of formula VII to amino groups, and

(vii) subjecting the diamino compound from step (vi) to ring closing conditions whereby a PBD dimer of formula I is obtained.

9. A method according to claim 8, in which step (vii) is carried out so as to leave an $-SC_2H_5$ substituent in the 11- and 11'- positions of the PBD dimer obtained (the nitrogen in the 10- and 10'-positions being in the amine form).

10. A method according to claim 8 or 9, in which the condensation reaction of step (i) is carried out by slowly adding a solution of the dihaloalkane III in an organic solvent to a two-phase reaction medium containing the benzoic acid derivative II, the reaction medium comprising an organic solvent which is the same as or miscible with the organic solvent present in the dihaloalkane III solution and an aqueous alkali.

11. A pharmaceutical composition comprising a PBD

dimer of formula I specified in claim 1 and an inert, physiologically acceptable carrier.

12. A pharmaceutical composition according to claim 1, in the form of an injectable solution, a formulation for oral administration, or a composition for topical application.

13. A method of treating cancer in a human or animal patient, which comprises the step of administering to said patient an effective amount of a pyrrolobenzodiazepine derivative as set forth in claim 1.

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 93/00483

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C07D519/00; A61K31/55; //(C07D519/00,487:00,487:00)
(C07D519/00,498/00,498/00)

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C07D ; A61K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
A	TETRAHEDRON LETTERS vol. 29, no. 40, 1988, OXFORD GB pages 5105 - 5108 J.W. SUGGS ET AL. 'Synthesis and DNA crosslinking ability of a dimeric anthramycin analog' cited in the application see complete article	1,14
P,X	JOURNAL OF THE CHEMICAL SOCIETY, CHEMICAL COMMUNICATIONS no. 20, 15 October 1992, LETCHWORTH GB pages 1518 - 1520 D.E. THURSTON ET AL. 'Effect of linker length on DNA-binding affinity, cross-linking efficiency and cytotoxicity of c8-linked pyrrolobenzodiazepine dimers' see complete article	1,11,14

* Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the International filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
23 JUNE 1993	30. 06. 93
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	ALFARO FAUS I.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB93/00483

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claim 16 is directed to a method of treatment of (diagnostic method practised on) the human/animal body, the search has been carried out and based on the alleged effects of the compound/compositon."

Remark: Claims 9 to 13 on pages 45 and 46 have been renumbered 12 to 16

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.